Plant Breeding **127**, 619—624 (2008)
doi:10.1111/j.1439-0523.2008.01545.x


# Lowering seed gossypol content in glanded cotton (*Gossypium hirsutum* L.) lines

G. B. Romano[1] and J. A. Scheffler[1,2]

[1]Crop Genetics and Production Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776, USA;
[2]Corresponding author, E-mail: jodi.scheffler@ars.usda.gov

*With 2 figures and 4 tables*

*Received October 31, 2007/Accepted June 11, 2008*
*Communicated by W. Friedt*

## Abstract

Cottonseed is a rich source of high quality protein, but its value as an animal feed is limited by gossypol, a toxic polyphenolic compound contained in glands located throughout the plant. This compound helps protect the plant from pests. Totally glandless varieties have been developed, but not adopted as these plants are left vulnerable to pests. This study describes a breeding strategy to decrease the levels of gossypol in the seed while maintaining a high enough concentration of toxin in vegetative plant parts to offer protection from pests. Preliminary studies indicated that crosses between varieties with different gland densities and distributions produced a range of glanding patterns. By selecting within the resulting progeny, we have identified $F_7$ generation progeny that have $<0.30\%$ total gossypol in the seed, while still possessing glands at critical locations on the vegetative plant parts. These new lines will be a valuable source of germplasm for developing low seed gossypol varieties. Seed from these varieties would provide a new source of inexpensive protein for animal feeding rations.

**Key words:** cotton — glanding — gossypol

Cotton (*Gossypium* spp.) is cultivated for its fibre (lint), but for every kg of lint produced 1.6 kg of seed is left as a by-product. Cotton seed is rich in oil (ca. 21%) and high quality protein (ca. 23%) (Lusas and Jividen 1987). However, the presence of a terpenoid compound called gossypol limits use of the protein. Gossypol accumulates in glands that are present throughout most of the vegetative and reproductive tissues of cotton plants (Adams et al. 1960). Gossypol and other terpenoids protect the plant from pests and possibly some diseases (Bottger et al. 1964, Bell and Stipanovic 1977, Hedin et al. 1992). Unfortunately, gossypol also has a detrimental effect on humans and monogastric animals and it is known to have anti-nutritional effects on warm-blooded animals and fish fed cottonseed products (Eisele 1986, Blom et al. 2001, Henry et al. 2001). Because of their special digestive system, adult ruminants can tolerate gossypol, and at present, cottonseed meal is mostly fed to adult ruminants, but only in limited quantities to prevent negative effects (Kim et al. 1996, Santos et al. 2003). A reduction in seed gossypol content would allow the increase in cottonseed meal in ruminant rations, and perhaps allow the expansion of its use by other species.

There is considerable natural variation for total seed gossypol content among *Gossypium* species (0.0–3.6%), and even among *G. hirsutum* L. cultivars it can range from 0.4% to 1.7% (Adams et al. 1960, Bell and Stipanovic 1977, Stipanovic et al. 2005). Processes to remove gossypol from cottonseed products exist (Mayorga et al. 1975); however, these treatments add cost to the products and reduce the nutritional value of the resulting cottonseed meal (Bell and Stipanovic 1977, Lusas and Jividen 1987). One strategy to remove gossypol has been to completely eliminate all gossypol glands on the plant. A glandless genetic stock was developed by McMichael (1959, 1960), and for the next 20 years extensive efforts were made to develop glandless cotton cultivars. These totally glandless cultivars have not been successful commercially because without the glands on the vegetative parts of the plant, they suffered increased damage from a number of pests (Jenkins et al. 1966, Hess 1977, Lusas and Jividen 1987). Other attempts to eliminate gossypol in seeds included the introgression of the glandless seed glanded plant trait into upland cotton from *G. sturtianum* Willis (Dilday 1986, Altman et al. 1987, Vroh Bi et al. 1999). However, there were some problems with sterility and lethality and no lines have been released.

While completely eliminating glands (and gossypol) has not been commercially viable, we hypothesized that a more moderate strategy might be successful. Although several minor genes may also affect glanding, it is generally accepted that there are two major genes, $Gl_2$ and $Gl_3$ (McMichael 1960). Lee crossed fully glanded ($Gl_2Gl_2Gl_3Gl_3$) cotton lines with glandless ($gl_2gl_2gl_3gl_3$) lines and evaluated glanding in the $F_2$ progeny (Lee 1962, 1965, 1977, 1978). In two studies, he also estimated seed gossypol content and reported that as the number of dominant alleles in the genotype decreased, so did the seed gossypol percentage (Lee et al. 1968, Lee 1977). Several studies have reported that the $gl_2gl_2Gl_3Gl_3$ genotype had a greater affect on decreasing seed glanding than $Gl_2Gl_2gl_3gl_3$, but the degree to which other plant organs were affected was not clear. Much of the confusion was due to the many different types of genetic material, organs and developmental stages used for the studies and the variety of analytical methods utilized to determine gossypol content (Rhyne et al. 1959, Lee 1962, 1965, 1978, Wilson and Lee 1976, Bell and Stipanovic 1977, Wilson and Smith 1977). Rhyne et al. (1959) reported that crosses between genotypes with glands and no glands produced progeny with a range of gland numbers and distribution.

The present study evaluated progeny from crosses between glanded (GL) × glandless (gl) parents to determine whether we could exploit this variation to develop genotypes with stably inherited lower seed gossypol, but near-normal glanding in the remainder of the plant. The study also evaluated different methods to determine which would most effectively identify desirable low seed gossypol genotypes or at least allow early elimination of unsuitable genotypes from the segregating progeny.

## Materials and Methods

The glandless parent 'STV 7A' gl is a $BC_5$ bulk of glandless progeny from a cross between 'Stoneville 7A' (STV 7A) and an unknown glandless line (W.R. Meredith Jr, USDA-ARS, personal communication). The glanded parents include 'Stoneville 7A', (Bowman et al. 2006), 'Maxxa', JaJo 6078 and A1006. 'Maxxa' is an Acala cultivar (Bowman et al. 2006) selected for this study because of its good fibre length and strength. JaJo 6078 is a smooth-leaved, nectariless line derived from a three-way cross of ($F_1$, LA 887/LA 850082FN) × ($F_1$, LA 887/MD51ne) (Jack Jones, JaJo Genetics, Baton Rouge, LA, USA). A1006 is a high fibre quality elite line from Australia's Commonwealth Scientific and Industrial Research Organisation.

Crosses were made between each of the glanded parents and STV 7A gl. These four populations were advanced to the $F_7$, $F_8$ or $F_9$ generations and various plant parts scored. The initial crosses were made in 1999 at Stoneville, Mississippi and 30 $F_1$ plants, from each cross combination, were grown at the Winter Nursery in Tecomán, Mexico, during the winter of 1999. In 2000, $F_2$ plants were grown in the field at Stoneville, and 60 plants from each cross were taken at random. The populations were designated STV, Maxxa, JaJo and A6. In 2001, $F_3$ progeny rows were grown at Stoneville. All plots in this study were single rows 4.5 m in length with 1.0 m between plots. From the 60 plots of the STV population, 40 plants that covered the full range of glanding distribution and density were selected and advanced to the $F_8$ generation. Results presented here are from the $F_7$ (2004) and $F_8$ (2005) generations. In 2005, 15 STV lines were selected based on plant glanding scores and seed gossypol content; these lines were evaluated in 2006 with two field replicates in single row plots. The STV 7A GL × STV 7A gl progeny were evaluated the most extensively as the parents were closely related and probably represented near-isogenic lines. This population provided the opportunity to observe the effect of 'glanding' genotype without confounding due to different genetic backgrounds.

The same procedure was followed for Maxxa and JaJo populations except that 20 $F_3$ plants each were selected for JaJo and Maxxa that covered the range of glanding distribution and density. The A6 population was advanced following a similar scheme except that 30 plants were selected from the $F_3$ progeny rows and it was advanced to an additional generation at the winter nursery. Results from the $F_7$ and $F_8$ generations for Maxxa and JaJo and $F_8$ and $F_9$ for A6 are presented here. These three populations were evaluated to test the usefulness of different types of measurements and to determine whether the selection strategy would be effective in different genetic backgrounds. In addition, a subset of plants was selected in 2005 based on plant glanding scores and per cent seed gossypol content and further evaluated in the field in 2006.

**Plant measurements:** For the STV population, gland presence and/or abundance were recorded for stems, stigmas, leaves, calyces and bolls. Total seed gossypol was evaluated by high-performance liquid chromatography (HPLC). These measurements were taken to identify whether glanding patterns on any plant tissue were closely associated with seed gossypol content and would provide an easy visual test to identify plants with few seed glands and low seed gossypol. The second purpose of the measurements was to characterize glanding patterns on plants with varying levels of seed gossypol to determine whether it was possible to identify genotypes with low gossypol and near-glandless seed that maintained glanding on the rest of the plant. Similar measurements were made for the JaJo, Maxxa and A6 populations.

The presence or absence of gossypol glands was assessed visually in stems and stigmas. The presence, absence and degree of glanding were evaluated in leaves, calyces and bolls. The parental plants were considered to have 'normal glanding', which meant there were glands on the leaf margins, veins and throughout the interveinal area; these were scored as 6. The rating system for leaves was 0 = glandless, 2 = very few glands on the interveinal region, 4 = reduced number of interveinal glands (intermediate between 2 and 6), and 6 = normal glanding with glands throughout the interveinal area and on the margins and veins. If categories 0, 2 or 4 had 'normal' glanding on the leaf margins or veins, 1.0 was added to the score. Counting the exact number of glands on leaf blades was not deemed necessary in the present study but special attention was paid to critical areas such as leaf margins and veins. There were no observed differences in the size of the glands, only a decrease in gland number. In 2004, six plants were scored per plot and in 2005 and 2006 three plants per plot were rated. The ratings were made by the same person and values presented are the mean of the plants scored.

Calyx glanding was scored from 0 to 4, with 0 = glandless, 1 = glands at the base only (peduncle), 2 = glands present from the base to the widest part of the flower bud, 3 = glands throughout the entire calyx except the calyx crown (sepal margins) and 4 = same as 3 plus glands along the margins of calyx crown. Eight plants were scored for each plot. The glanding scale used for bolls was 0 = glandless; 1 = glands in the base of the boll; 2 = band of glands parallel to boll sutures, the central portion of each carpel lacks glands; 3 = glands throughout the entire surface of the boll; 4 = distribution of glands as in 3, but in higher numbers and the surface of the boll appears pitted where glands occur. Six plants per plot were scored in 2004 and three plants per plot were rated in 2005 and 2006.

**Gossypol analysis:** Sixteen seeds were soaked for 16 h at 25°C, manually de-hulled, freeze-dried and ground in a tissue pulverizer. The protocol used to quantify (+) or (−) gossypol and total gossypol followed Hron et al. (1999) with several modifications. For each extraction, approximately 100 mg of powdered sample was weighed into a KIMAX glass tube (exact weight was recorded and used in gossypol per cent calculations). Two millilitres of complexing reagent (2% (R)-(-)-2-amino-propanol, 10% glacial acetic acid, 88% *N*,*N*-dimethyl formamide) was added to each tube.

The samples were incubated at 100°C for 30 min and cooled to room temperature. Eight millilitres of isocratic mobile phase (85% acetonitrile and 15% phosphate buffer, 0.01 M $KH_2PO_4$, adjusted to pH 3.0 with $H_3PO_4$) was added to each tube and mixed. Particles were allowed to settle and part of the supernatant was transferred into a microfuge tube. The microfuge tubes were centrifuged for 2 min at 11 269 ***g*** to pellet any remaining particles, and the supernatant transferred to HPLC vials. Samples were analysed in an Agilent (Santa Clara, CA, USA) series 1100 HPLC. A reverse phase SGE Inertsil ODS-2 cartridge column (5 μm, 100 × 4.0 mm i.d.) was used and the diode array detector (model G1315A) was set to 254 mm. Flow rate was 1 ml/min and the injection volume was 20 μl/sample. Retention times for (+) and (−) gossypol derivatives were approximately 1.8 and 2.8 min respectively.

## Results

In 2004, stem and stigma glanding were evaluated in the STV population ($F_7$). Plants with glandless stems and stigmas were found to be totally glandless with 0% seed gossypol. Per cent total seed gossypol for plants with stem and stigma glands varied from 0.1% to 2.0% in 2004 ($F_7$) and 0.1% to 1.8% in 2005 ($F_8$), indicating that stem and stigma glanding were not good predictors of seed glanding or gossypol content. Similar results were obtained for the JaJo, Maxxa and A6 populations. However, scoring stem glanding proved to be an easy method to eliminate glandless genotypes from the populations.

The correlation between boll glanding score and per cent seed gossypol for the STV population was significant although not high enough to prevent selection for moderate boll glanding and low seed gossypol (Table 1). This enabled the selection of lines with gossypol content as low as 0.36%, but with boll glanding along the critical points at the base and sutures of the boll (Fig. 1). Similar results were obtained for the JaJo, Maxxa and A6 populations (Fig. 2). In the STV

Table 1: Pearson correlation coefficients for glanding scores in stigmas, calices, stems, bolls, and total per cent gossypol in seeds for the STV population in 2004 ($F_7$) and 2005 ($F_8$)

| | Year | Stigma | Calyx | Stem | Boll |
|---|---|---|---|---|---|
| Stem | 2004 | 0.93 | n/a | – | – |
| | 2005 | n/a | 0.85 | – | – |
| Boll | 2004 | 0.27 | n/a | 0.27 | – |
| | 2005 | n/a | 0.54 | 0.23 | – |
| % Seed gossypol | 2004 | 0.47 | n/a | 0.47 | 0.76 |
| | 2005 | n/a | 0.63 | 0.47 | 0.76 |

All coefficients are P = 0.01. n/a, not scored.

Table 2: Pearson correlation coefficients comparing per cent total gossypol in seed from the $F_7$ and $F_8$ generations for the STV, JaJo and Maxxa populations, and $F_8$ and $F_9$ for the A6 population (P = 0.01)

| | Pearson correlation coefficients of total seed gossypol | |
|---|---|---|
| | *r* | n |
| STV | 0.97 | 120 |
| JaJo | 0.89 | 45 |
| Maxxa | 0.94 | 41 |
| A1006 | 0.98 | 45 |

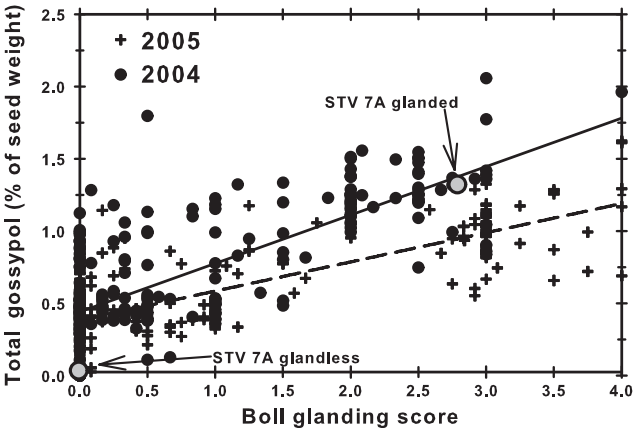


Fig. 1: Total seed gossypol (% of seed weight) and mean boll glanding scores in 2004 ($F_7$) and 2005 ($F_8$) for the STV population. The solid line is the 2004 regression line ($r$ = 0.76, P = 0.01) and the dashed line is the 2005 regression line ($r$ = 0.76, P = 0.01)

population, calyx glanding was correlated with boll glanding and total seed gossypol; however, both correlation coefficients were moderate (Table 1) indicating that it would be possible to select relatively high scores for calyx glanding while keeping seed gossypol content low (Table 4).

Analysis of variance (ANOVA) calculated for the STV population, showed significant line and year effects (P = 0.01), but line × replication and line × year interactions were not significant (P = 1.00), indicating that seed gossypol content was uniformly affected in all lines by environmental factors. In general, total seed gossypol content was stable in the $F_7$ and $F_8$ progeny in all populations (Table 2). The lines were also ranked by gossypol content in both generations and the rankings were found to be correlated in all populations (r = 0.95 STV, r = 0.77 JaJo, r = 0.92 Maxxa and r = 0.95 A6; all P = 0.01). Closer inspection of the data indicated that lines with seed gossypol content higher than 0.5% did not vary in their rankings in 2004 and 2005 but a few of the values varied by as much as 0.5%. For lines with seed gossypol levels below 0.5%, neither the rankings nor the actual values changed significantly over years. As only a single plant was advanced in each generation, some of the lines could still be segregating, and the variation observed could have been due to selection of plants with different genotypes. In any case, it emphasizes that lines need to be evaluated over years to test for stability. Examination of the (+) and (−) gossypol values showed that all the lines had the expected 60 : 40 ratio of (+) to (−) gossypol.

Earlier reports (Lee 1962, 1965, Rhyne 1962) indicated that the monomeric genotypes $gl_2gl_2GL_3GL_3$ and $GL_2GL_2gl_3gl_3$ exhibited normal glanding in leaves early in plant development, but, as the season progressed, newly emerged leaves exhibited decreased glanding. The number of glands on each leaf is constant from leaf emergence to senescence and glands are located on main veins and on the interveinal areas. Leaf glanding was evaluated on the subset of 27 selected lines from

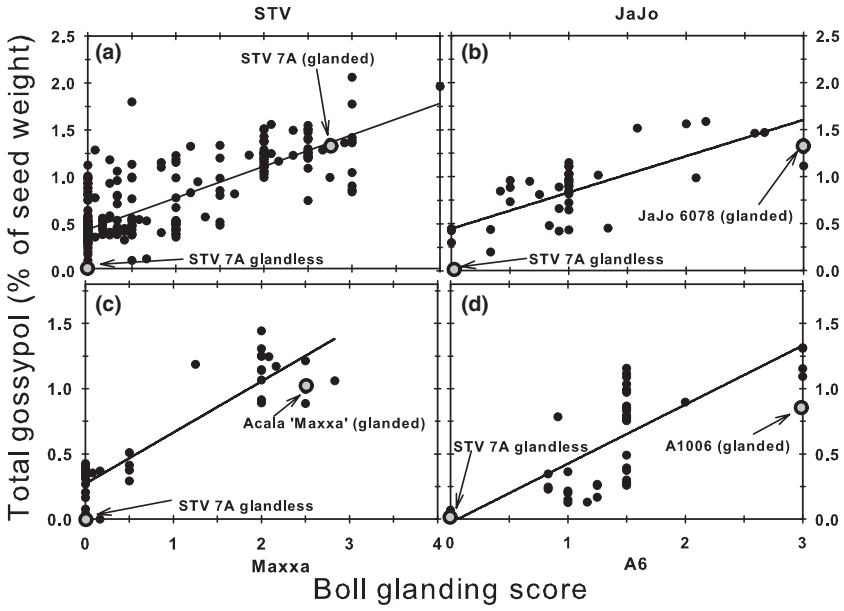


Fig. 2: Total seed gossypol (% of seed weight) and mean boll glanding scores from 2004 for STV, JaJo, Maxxa and A6 populations. The correlation coefficients are: a – STV $r$ = 0.76, P = 0.01; b – JaJo $r$ = 0.75, P = 0.01; c – Maxxa $r$ = 0.91, P = 0.01 and d – A6 $r$ = 0.70, P = 0.01

Table 3: Glanding in the five parents and 27 lines selected from the STV, JaJo, Maxxa and A6 populations grown in the field in 2006

| Line | Stem glanding[1] | Leaf glanding at flowering[2] | Leaf glanding at cut out[3] | Calyx glanding[4] | Boll glanding[5] |
|---|---|---|---|---|---|
| STV 7A | 1.0 | 6.0 | 6.0 | 3.0 | 2.5 |
| JaJo 6078 | 1.0 | 6.0 | 6.0 | 3.0 | 3.0 |
| Acala 'Maxxa' | 1.0 | 6.0 | 6.0 | 3.0 | 2.5 |
| A1006 | 1.0 | 6.0 | 6.0 | 3.0 | 3.0 |
| STV 7A gl | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| STV-1 | 1.0 | 5.7 | 1.3 | 1.7 | 0.2 |
| STV-2 | 1.0 | 5.2 | 2.7 | 2.7 | 0.3 |
| STV-3 | 1.0 | 4.7 | 1.0 | 3.8 | 3.2 |
| STV-4 | 1.0 | 3.7 | 1.3 | 3.0 | 1.4 |
| STV-5 | 1.0 | 6.0 | 5.7 | 3.0 | 3.5 |
| STV-6 | 1.0 | 5.0 | 1.0 | 2.2 | 1.3 |
| STV-7 | 1.0 | 4.7 | 2.3 | 2.8 | 0.9 |
| STV-8 | 1.0 | 4.3 | 2.7 | 2.8 | 0.8 |
| STV-9 | 1.0 | 5.0 | 2.3 | 2.8 | 0.8 |
| STV-10 | 1.0 | 5.3 | 2.7 | 2.8 | 1.3 |
| STV-11 | 1.0 | 5.0 | 1.3 | 2.4 | 0.3 |
| STV-12 | 1.0 | 4.7 | 2.0 | 2.4 | 1.6 |
| STV-13 | 1.0 | 5.0 | 2.7 | 2.5 | 0.4 |
| STV-14 | 1.0 | 5.3 | 3.0 | 3.0 | 1.2 |
| STV-15 | 1.0 | 4.2 | 2.0 | 2.3 | 0.8 |
| JAJO-16 | 1.0 | 6.0 | 1.3 | 2.9 | 0.0 |
| JAJO-17 | 1.0 | 6.0 | 3.0 | 3.0 | 0.0 |
| JAJO-18 | 1.0 | 6.0 | 3.0 | 2.8 | 1.8 |
| MAXXA-19 | 1.0 | 4.3 | 1.0 | 2.2 | 0.1 |
| MAXXA-20 | 1.0 | 4.5 | 1.3 | 2.4 | 1.0 |
| MAXXA-21 | 1.0 | 6.0 | 3.0 | 2.7 | 1.7 |
| MAXXA-22 | 1.0 | 6.0 | 6.0 | 4.0 | 3.5 |
| MAXXA-23 | 1.0 | 3.3 | 1.0 | 2.0 | 0.2 |
| MAXXA-24 | 1.0 | 5.0 | 1.3 | 2.8 | 0.2 |
| A1006-25 | 1.0 | 5.0 | 1.0 | 2.0 | 0.0 |
| A1006-26 | 1.0 | 4.8 | 1.0 | 3.0 | 1.6 |
| A1006-27 | 1.0 | 4.3 | 1.0 | 2.3 | 0.0 |

Values are the means of three plants sampled in each of two replications.
[1]0 = no glands, 1 = normal glanding.
[2]0 = no glands, 6 = normal glanding.
[3]0 = no glands, 6 = normal glanding.
[4]0 = no glands, 3 = normal glanding, 4 = glanding up to calyx margin (crown). Mean of two replicates of eight plants each.
[5]0 = no glands, 3 = normal glanding, 4 = dense glanding. Bolls < 1 week old.

Table 4: Glanding and per cent total seed gossypol for the five parents and six lines selected from the STV, JaJo, Maxxa and A6 populations grown in the field in 2006. These lines were selected based on their optimum combination of plant glanding and seed gossypol

| Population | Line | Stem glanding[1] | Leaf glanding at flowering[2] | Leaf glanding at cut out[3] | Calyx glanding[4] | Boll glanding[5] | % Total gossypol |
|---|---|---|---|---|---|---|---|
| STV | 3 | 1.0 | 4.7 | 1.0 | 3.8 | 3.2 | 0.738 |
| STV | 4 | 1.0 | 3.7 | 1.3 | 3.0 | 1.4 | 0.364 |
| STV | 10 | 1.0 | 5.3 | 2.7 | 2.8 | 1.3 | 0.377 |
| JaJo | 18 | 1.0 | 6.0 | 3.0 | 2.8 | 1.8 | 0.421 |
| Maxxa | 21 | 1.0 | 6.0 | 3.0 | 2.7 | 1.7 | 0.555 |
| A6 | 26 | 1.0 | 4.8 | 1.0 | 3.0 | 1.6 | 0.276 |
| STV 7A GL | Parent | 1.0 | 6.0 | 6.0 | 3.0 | 2.5 | 1.296 |
| JaJo | Parent | 1.0 | 6.0 | 6.0 | 3.0 | 3.0 | 1.360 |
| Maxxa | Parent | 1.0 | 6.0 | 6.0 | 3.0 | 2.5 | 0.885 |
| A6 | Parent | 1.0 | 6.0 | 6.0 | 3.0 | 3.0 | 0.968 |
| STV 7A gl | Parent | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | ND |

Values are the means of three plants sampled in each of two replications. ND, not detected.
[1]0 = no glands, 1 = normal glanding.
[2]0 = no glands, 6 = normal glanding.
[3]0 = no glands, 6 = normal glanding.
[4]0 = no glands, 3 = normal glanding, 4 = glanding up to calyx margin (crown). Mean of two replicates of eight plants each.
[5]0 = no glands, 3 = normal glanding, 4 = dense glanding. Bolls < 1 week old.

the four populations in 2006 to determine whether the lines still had glands on the leaves (Table 3). Glanding was recorded at the time the first bloom appeared and when plants stopped producing bolls (cut out). Up to flowering, the plants had fully glanded leaves but the number of glands on new leaves gradually decreased until cut out. In this study, all the selected lines had leaves with glands at the critical points on their margins and veins until cut out. Six lines were selected for further testing based on their combination of glanding properties and total gossypol content (Table 4).

## Discussion

The moderate correlation between seed gossypol content and boll glanding scores allowed for selection of lines with seed gossypol content of as low as 0.36%, a boll score of 1.4 and a normal calyx glanding score of 3 (line STV 4, Table 4). Calyx glands have been shown to protect the bud from pests such as bollworms (Lukefahr and Martin 1966, Parrott et al. 1989, Calhoun 1997); therefore, it was important to maintain normal calyx glanding while reducing the percentage of seed gossypol. All the parental lines used in this study had glands throughout the calyx except on the calyx crown. This is the typical glanding pattern for upland cotton; however, there are some unique lines that have glands on the calyx crown and these are thought to enhance the plant's resistance to pests (Parrott et al. 1989, Calhoun 1997). All the 2006 selections had normal calyx glanding (Table 4).

There was often a reduction in leaf glanding as the growing season progressed. That is, lines had near-normal glanding at first bloom, but at cut out terminal leaves had glands only on the margins and veins. In all these selections, even at the end of the growing season the leaves were protected by glands in the critical areas along the leaf margins and veins. No reduction in gland size was observed. Only plants glandless in all other organs had totally glandless leaves. These results agree with a study by Rhyne (1962) where he reported that only plants with the genotype $gl_2gl_2gl_3gl_3$ had totally glandless leaves. Overall, STV 10 had the best combination of plant glanding and seed gossypol (0.38%, Table 4).

Some of the lines had higher levels of seed gossypol than the glanded parent (Figs 1 and 2). For the JaJo, Maxxa and A6 populations, the progeny with seed gossypol higher than their glanded parent might be due to complementary genes contributed by the glandless parent. For the STV population, this should not be the case if the GL and gl parents were true near-isogenic lines. We cannot verify that the STV 7A GL line used here was from the same source as the STV 7A used to develop the STV 7A gl line. Moreover, it has been documented that cotton cultivars can be heterogeneous and can change over time. Our analysis of STV 7A from a different source showed a per cent total seed gossypol of 1.56% and the source we used in this study ranged from 1.30% to 1.38%.

Although boll and calyx glanding were correlated with per cent of gossypol in seed, the correlation coefficients were not high enough to make selections based on either of these visual scores. Ultimately only a combination of plant glanding evaluation and direct quantification of seed gossypol was adequate for successful selection. To allow analysis of the large number of samples required for this type of selection, it was essential to use a gossypol quantification method that could handle numerous samples and minimize cost without sacrificing accuracy; the HPLC method used in this study accomplished both.

Seed gossypol content was highly correlated in all lines in consecutive years and seed gossypol percentage in STV lines showed no interaction with planting year. Pons et al. (1953) evaluated eight varieties at 13 locations over 3 years and reported a significant year effect, but noted that the rankings of the varieties did not change over years. In this study, the lines of interest with seed gossypol $< 0.5\%$ did not vary over years. As the number of glands is highly heritable and not affected by environment (Bell and Stipanovic 1977), the stability of gossypol content, in the low seed gossypol lines described here, may be due to the fact that these lines have a consistently low number of seed glands available to act as storage sites for the gossypol. Although the exact size of the seed glands was not measured, there was no observed reduction in gland size of the low gossypol lines.

Sunilkumar et al. (2006) recently reported using iRNA techniques to produce $F_2$ transgenic plants with 0.1 μg/mg seed gossypol, while maintaining gossypol and related terpenoids in the foliage and floral parts of the plant. This technique represents a possible new method to modify seed gossypol, but further testing is needed, and the lines will face all the regulatory hurdles that are in place for commercializing transgenic plants. Finally it must gain public acceptance before it can be used as a food source. Our lines are developed by conventional breeding techniques and can avoid the regulatory restrictions and the public aversion to genetically modified organisms (GMOs).

This study demonstrated that it was possible to select for genotypes that minimized the seed gossypol content while preserving glanding in stems, bolls and calyces. The reduction by $> 50\%$ of per cent total seed gossypol compared with the glanded parent and the development of lines with 0.3% total seed gossypol will permit increased use of cottonseed meal in bovine feed rations and perhaps expand its use to monogastric species. Because cotton is grown for its fibre, not its seed, fibre yield is still the most important consideration when determining the value of a cultivar. This means that throughout the development process, evaluation of yield and fibre quality cannot be neglected. The six selections described in this paper (Table 4) are being further evaluated for seed gossypol and plant glanding, tested in yield trials, analysed for fibre quality and field tested for pest resistance. The next generation of semi-glanded plants are currently being developed using a glandless parent with good agronomic traits and crossing it to a glanded parent with the lowest seed gossypol possible.

## Acknowledgements

The authors are indebted to Michael Dowd for allowing us to use his unpublished protocol for HPLC analysis, to Scott Pelitire for analysing a portion of the samples and successfully transferring the HPLC assay method to our laboratory, to Teheshia Brooks, Gus Eifling, Shenita Harper, Shirley Howard and Robin Jordan for valuable technical assistance, to Debbie Boykin for statistical advice and to William R. Meredith Jr. for allowing selection of the $F_2$ plants from four of his populations to use in this study. We also thank Drs Jack Jones, Jack McCarty Jr, William Meredith Jr and Joshua Lee for their critical review of this manuscript.

## References

Adams, R., T. A. Geissman, and J. D. Edwards, 1960: Gossypol, a pigment of cottonseed. Chem. Rev. **60,** 555—574.

Altman, D. W., D. M. Stelly, and R. J. Kohel, 1987: Introgression of the glanded-plant and glandless-seed trait from *Gossypium sturtianum* Willis into cultivated upland cotton using ovule culture. Crop Sci. **27,** 880—884.

Bell, A. A., and R. D. Stipanovic, 1977: The chemical composition, biological activity, and genetics of pigment glands in cotton. Proceedings of the Beltwide Cotton Conferences, Atlanta, GA, USA, 244—258.

Blom, J. H., K.-J. Lee, J. Rinchard, K. Dabrowski, and J. Ottobre, 2001: Reproductive efficiency and maternal-offspring transfer of gossypol in rainbow trout (*Oncorhynchus mykiss*) fed diets containing cottonseed meal. J. Anim. Sci. **79,** 1533—1539.

Bottger, G. T., E. T. Shehan, and M. J. Lukefahr, 1964: Relation of gossypol content of cotton plants to insect resistance. J. Econ. Entomol. **57,** 183—185.

Bowman, D. T., O. A. Gutierrez, R. G. Percy, D. S. Calhoun, and O. L. May, 2006: Pedigrees of Upland and Pima Cotton Cultivars Released between 1970 and 2005. Bulletin 1155. Mississippi Agricultural and Forestry Experiment Station, Stoneville, MS.

Calhoun, D. S., 1997: Inheritance of high glanding, an insect resistance trait in cotton. Crop Sci. **37,** 1181—1186.

Dilday, R. H., 1986: Development of a cotton plant with glandless seeds, and a glanded foliage and fruiting form. Crop Sci. **26,** 639—640.

Eisele, G. R., 1986: A perspective on gossypol ingestion in swine. Vet. Hum. Toxicol. **28,** 118—122.

Hedin, P. A., W. L. Parrott, and J. N. Jenkins, 1992: Relationships of glands, cotton square terpenoid aldehydes, and other allelochemicals to larval growth of *Heliothis virescens* (Lepidoptera: Noctuidae). J. Econ. Entomol. **85,** 359—364.

Henry, M. H., G. H. Pesti, and T. P. Brown, 2001: Pathology and histopathology of gossypol toxicity in broiler chicks. Avian Dis. **45,** 598—604.

Hess, D. C., 1977: Genetic improvement of gossypol-free cotton varieties. Cereal Food World **22,** 98—103.

Hron, R. J., Sr, H. L. Kim, M. C. Calhoun, and G. S. Fisher, 1999: Determination of (+)-, (−)-, and total gossypol in cottonseed by high-performance liquid chromatography. AOCS **76,** 1351—1355.

Jenkins, J. N., F. G. Maxwell, and H. N. Lafever, 1966: The comparative preference of insects for glanded and glandless cottons. J. Econ. Entomol. **59,** 352—355.

Kim, H. L., M. C. Calhoun, and R. D. Stipanovic, 1996: Accumulation of gossypol enantiomers in ovine tissues. Comp. Biochem. Physiol. **113,** 417—420.

Lee, J. A., 1962: Genetical studies concerning the distribution of pigment glands in the cotyledons and leaves of upland cotton. Genetics **47,** 131—142.

Lee, J. A., 1965: The genomic allocation of the principal foliar-gland loci in *Gossypium hirsutum* and *Gossypium barbadense*. Evolution **19,** 182—188.

Lee, J. A., 1977: Inheritance of gossypol level in *Gossypium* III: genetic potentials of two strains of *Gossypium hirsutum* L. differing widely in seed gossypol level. Crop Sci. **17,** 827—930.

Lee, J. A., 1978: Inheritance of gossypol level in *Gossypium* IV. Results from the reciprocal exchange of the major gossypol-gland alleles between *G. hirsutum* L. and *G. barbadense* L. Crop Sci. **18,** 482—484.

Lee, J. A., C. C. Cockerham, and F. H. Smith, 1968: The inheritance of gossypol level in *Gossypium*. I. Additive, dominance, epistatic and maternal effects associated with seed gossypol in two varieties of *Gossypium hirsutum* L. Genetics **59,** 285—298.

Lukefahr, M. J., and D. F. Martin, 1966: Cotton pigments as a source of resistance to bollworm and tobacco budworm. J. Econ. Entomol. **59,** 176—179.

Lusas, E. W., and G. M. Jividen, 1987: Glandless cottonseed: a review of the first 25 years of processing and utilization research. AOCS **64,** 839—854.

Mayorga, H., J. Gonzaléz, J. F. Menchu, and C. Rolz, 1975: Preparation of a low free gossypol cottonseed flour by dry and continuous process. J. Food Sci. **40,** 1270—1274.

McMichael, S. C., 1959: Hopi cotton, a source of cotton-seed free of gossypol pigments. Agron. J. **51,** 630.

McMichael, S. C., 1960: Combined effects of glandless genes $gl_2$ and $gl_3$ on pigments in the cotton plant. Agron. J. **52,** 385—396.

Parrott, W. L., J. N. Jenkins, J. E. Mulrooney, J. C. McCarty, and R. L. Shepard, 1989: Relationship between gossypol gland density on cotton squares and resistance to tobacco budworm larvae. J. Econ. Entomol. **82,** 589—592.

Pons, W. A., Jr, C. L. Hoffpauir, and T. H. Hopper, 1953: Gossypol in cottonseeds. Influence of variety of cottonseed and environment. J. Agric. Res. Food Chem. **1,** 1115—1118.

Rhyne, C. L., 1962: Inheritance of the glandless-leaf phenotype in upland cotton. J. Hered. **53,** 115—123.

Rhyne, C. L., F. H. Smith, and P. A. Miller, 1959: The glandless leaf phenotype in cotton and its association with low gossypol content in the seed. Agron. J. **51,** 148—152.

Santos, J. E., M. Villasenor, P. H. Robinson, E. J. DePeters, and C. A. Holmberg, 2003: Type of cottonseed and level of gossypol in diets of lactating dairy cows: plasma gossypol, health, and reproductive performance. J. Dairy Sci. **86,** 892—905.

Stipanovic, R. D., L. S. Puckhaber, A. A. Bell, A. E. Percival, and J. Jacobs, 2005: Occurrence of (+)- and (−)-gossypol in wild species of cotton and in *Gossypium hirsutum* Var. *marie-galante* (Watt) Hutchinson. J. Agric. Food. Chem. **53,** 6266—6271.

Sunilkumar, G., L. M. Campbell, L. Puckhaber, R. D. Stipanovic, and K. S. Rathore, 2006: Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proc. Natl Acad. Sci. U.S.A. **103,** 18054—18059.

Vroh Bi, I., J. P. Baudoin, B. Hau, and G. Mergeai, 1999: Development of high-gossypol cotton plants with low-gossypol seeds using trispecies bridge crosses and in vitro culture of seed embryos. Euphytica **106,** 243—251.

Wilson, F. D., and J. A. Lee, 1976: Interrelationships among gland density, gossypol content, and lint and seed characters in cotton. Crop Sci. **16,** 860—861.

Wilson, F. D., and J. N. Smith, 1977: Variable expressivity and gene action of gland-determining alleles in *Gossypium hirsutum* L. Crop Sci. **17,** 539—543.